A number of possibilities might account for this situation. One, a strange sulfur compound might be present; two, a difficultly hydrolyzable peptide of cystine might have remained in the hydrolysate which would react in the Folin-Looney reaction but not in the Sullivan method; three, the cystine might be partially destroyed during hydrolysis; and, four, some substance might be generated during the hydrolysis which would account for the high Folin-Looney reaction; and, finally, various combinations of these possibilities might exist.

One of the first things we did when we undertook to settle this question was to heat various amino acids with hydrochloric acid and sulfuric acid to see if such treatment would produce substances that would be chromogenic with the Folin-Marenzi method, which was a modification of the Folin-Looney procedure.

Interestingly enough, methionine upon being heated with strong sulfuric acid was found to give a positive reaction with the Folin-Marenzi reagent for cystine. This was the start of our series of investigations concerning homocystine. In searching for the substance in the reaction mixture responsible for the positive test, we isolated a crystalline compound which we were able to demonstrate to be the next higher symmetrical homologue of cystine. The possibility of this compound being involved in the intermediary metabolism of methionine in the body immediately occurred to us. Investigations concerning the utilization of the homocystine by animals on a cystine-deficient diet and studies of the oxidation of the compound were therefore undertaken. Synthesis of the homocystine, resolution of it into its optical isomers, and the demonstration of the steric relationship between its isomers and those of methionine soon followed, as well as studies of the higher homologues of homocystine and methionine.

Although this observation of the effect of sulfuric acid on methionine led to some rather interesting results, it did not explain the particular thing we were after. In the first place, the difference between these reactions existed in hydrochloric acid hydrolysates, and hydrochloric acid did not yield a chromogenic substance from methionine; and, secondly, methionine was not present in insulin in more than traces, if at all. We still had the original question to solve, and we worked off and on on this problem during the next few years and just within the past few months we have obtained results which lead us to believe that we have finally accounted for the sulfur of insulin.

It is rather amusing that the entire homocystine work with which we have been engaged during the past six years would not have taken place had we been able to account for the sulfur of insulin at

that time. It is indeed curious the path which research may take, and perhaps it is these queer turns and quirks that make it fun to try to follow the pathway.

Although this particular path of homocystine researches appeared to have no connection with insulin, a recent turn of events has brought the two fields back together again and serves as an interesting example of how offshoots of a research may wander away and bend back again, touch, and even aid the original research. I refer here to the recent work on the question of whether or not a trace of methionine is present in crystalline insulin, and to the fact that one of the methods for determining methionine depends on the determination of the homocysteine thiolactone formed from methionine by hydriodic acid. I shall refer to this again later.

Along with attempts to fractionate insulin hydrolysates to see if a strange sulfur compound were present, and testing for the presence or absence of known sulfur-containing compounds such as thiolhistidine, we have also studied the question of the completeness of hydrolysis of insulin and the prevention of destruction of the cystine during hydrolysis.

To make a very long story short, after trying various procedures, we finally found that if we hydrolyzed insulin with twenty per cent hydrochloric acid and 50 per cent formic acid we were able to account by the Sullivan method for all of the sulfur as cystine within the experimental error of the method. We were able, furthermore, to adduce evidence that the previous low results were really due to a destruction of the cystine on the one hand and incomplete hydrolysis on the other.

Whether or not a trace of methionine is present cannot be stated definitely. Our own results on the study would indicate that if it is present at all it is even less than that reported by Brand. You can readily understand the difficulty of proving the presence or absence of a very slight trace of methionine in dealing with such a compound as insulin. One is dealing with such small amounts that one is at the borderline of the accuracy of the methods. Much more work will be needed before reaching a final decision.

Another aspect of the sulfur of insulin that has intrigued us, which even more forcibly brings out the importance of the sulfur, is the effect of reduction upon the activity of insulin. Earlier work had shown that various reducing agents destroyed the activity, but the reagents used were quite vigorous ones and one would have reason to believe that groupings other than the disulfide may have been re-